EPA Reviewer: Steven L. Malish, Ph.D., ____________ Team 1 RASSB/Antimicrobials Division (7510C)
Secondary Reviewer: Jonathan Chen, Ph.D., Team 3, ____________
RASSB/Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat; OPPTS 870.3700 [§83-3a]; OECD 414.

DP BARCODE:	D301664	**DECISION No.**	217014
PC CODE:	004115	**REGISTRATION No.**	59825R

TEST MATERIAL (PURITY): Tetraacetylethylenediamine (TAED, 99.4% a.i.)

SYNONYMS: N,N'-1,2-ethanediylbis[N-acetylacetamide], N,N'-ethylenebis[N-acetylacetamide] *N,N,N',N'*-Teraacetylethylendiamine, TAED

CITATION: Bussi, R. (1994). Prenatal Development Toxicity Study of Tetraacetyl-ethylenediamine in Rats via Oral Route. Instituto Di Richerche Biomedice "Antoine Marxer" RBM S.p.A. (Italy). RBM Experiment No. 920887, March 4, 1994. MRID 45299704. Unpublished.

SPONSOR: Warwick International, Limited
Mostyn, Holywell, Flintshire, CH8 9HE, United Kingdom

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 45299704), tetraacetyl-ethylenediamine (TAED) (99.4% a.i., batch 071292 [07.12.92]) was administered to 25 mated female Crl:CD (SD) BR rats/dose by gavage at dose levels of 0, 40, 200, or 1000 mg/kg/day from days 6 through 15 of gestation. Maternal toxicity was observed at 1000 and 200 mg/kg/day. Treatment-related effects at 1000 mg/kg/day included decreased body weight, body weight gain, corrected body weight, corrected body weight gain, and food consumption. Treatment-related effects at 200 mg/kg/day were similar to those observed at the high dose and included decreased body weight gain, corrected body weight gain, and food consumption. **The maternal LOAEL is 200 mg/kg bw/day, based on a reduction in body weight gain, corrected body weight gain, and food consumption. The maternal NOAEL is 40 mg/kg bw/day.**

Fetal toxicity was observed at 1000 mg/kg/day, with treatment-related effects including decreased mean fetal weights (male, female, and combined) and an increased

incidence of skeletal variations. There were no treatment-related effects observed at 200 or 40 mg/kg/day. **The developmental LOAEL is 1000, based on decreased mean fetal weights and increased incidence of skeletal variations. The developmental NOAEL is 200 mg/kg/day.**
This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a developmental toxicity study (OPPTS 870.3700 [§83-3a]; OECD 414) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements are provided in both studies.

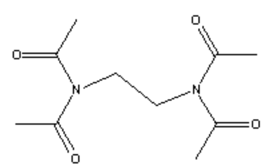
I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: TAED

Description:	White powder
Lot/Batch #:	Batch 071292 (07.12.92)
Purity:	99.4 % a.i.
Compound Stability:	3 years (20° C)
CAS #:	10543-57-4

Structure:



2. Vehicle and/or positive control: 1% carboxymethylcellulose in bi-distilled (double-distilled) water (Batch # 314337/1); purity was not provided

3. Test animals:

Species:	Rats
Strain:	Crl:CD (SD) BR
Age/weight at study initiation:	Approximately 9-10 weeks old and 200-225 g on receipt.
Source:	Females were supplied by Charles River Italia S.p.A. (Como)
Housing:	Animals of the same sex were housed two per cage prior to mating in Makrolon cages, each fitted with a stainless-steel cover-feed rack. After the mating period, gravid females were individually-housed.
Diet:	Animals were fed *ad libitum* a diet coded as GLP 4 25, top certificate, which reportedly did not contain levels of contaminants that would interfere with the study.

Water:	Filtered water from the municipal water main was provided *ad libitum*. Water was periodically analyzed for microbiological count, heavy metals, other contaminants (e.g., solvents, pesticides) and other physical and chemical characteristics and did not contain levels of contaminants that would interfere with the study.	
Environmental conditions:	**Temperature:**	22±2° C
	Humidity:	60±20%
	Air changes:	10-15 air changes/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Approximately 2 weeks	

B. PROCEDURES AND STUDY DESIGN

1. In life dates:

Arrival: January 22, 1993
Mating Start: February 8, 1993
Dosing Start: February 15, 1993
Necropsy: March 1-22, 1993

2. Mating: Male cages were alternated in close proximity to the female cages at the start of the mating period. One male was then placed in a cage containing 2 females every evening (4 evenings/week) for 16 hours. Every morning, females were examined for evidence of mating (presence of sperm in vaginal smear). The day on which sperm was detected in the vaginal smear was designated as gestation day (gd) 0.

3. Animal Assignment: Animal assignment is presented below in Table 1. Females exhibiting evidence of mating each morning were assigned to treatment groups, one per group by increasing group number. The same process was resumed the next day (beginning where it was left off). In order to ensure that 20 gravid dams were in each group, 25 copulated females were allocated to each of the four dose groups. There is no indication that a formal randomization procedure was used or that any attempt was made to equalize initial group body weights.

TABLE 1. Animal Assignment

Dose (mg/kg bw/day)	0	40	200	1000
#Mated females	25	25	25	25

4. Dose selection rationale: The dose levels selected for this study were based on data from acute and subacute toxicity studies in which dosages of 0, 40, 200, and 1000 mg/kg/day were administered to rats. Information regarding the specific study number and results of the these studies are not provided in the study report.

5. Dosage preparation and analysis: The test material was suspended in the required amount of vehicle to obtain concentrations of 4, 20, and 100 mg/mL, formulations were prepared as needed and stirred and protected from light until use. The concentration of the test material-vehicle mixtures was evaluated by RBM (study laboratory) for those mixtures prepared on February 18, 1993. It was not reported whether the test material-vehicle mixtures were evaluated for stability and/or homogeneity.

Results

Homogeneity Analysis: It was not reported whether the test material-vehicle mixtures were evaluated for homogeneity.

Stability Analysis: It was not reported whether the test material-vehicle mixtures were evaluated for stability.

Concentration Analysis: According to the study report, the concentrations of the test material-vehicle mixtures were within the expected values. No data are provided to support this statement.

The study report does not provide any analytical data from the concentration analysis; consequently, it is unknown whether the procedures used were adequate or whether the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration: All doses were administered by oral gavage once daily on gd 6 through 15 in a volume of 10 mL/kg of body weight/day. The exact volume administered was based on the most recent body weight determination.

C. OBSERVATIONS

1. Maternal Observations and Evaluations: Rats were observed daily for physical appearance, behavior, and clinical signs; during treatment, dams were observed for any possible reaction to treatment. Body weights were recorded on gd 0, 6, 10, 15, 18, and 20 and were used to calculate mean body weight gain and corrected body weight gain (final body weight excluding gravid uterine weight). Food consumption (g/animal/day) was determined for gd 6, 10, 15, 18, and 20.

Dams were sacrificed on gd 20 by cervical dislocation after anesthetization with carbon dioxide. Dams were subjected to a gross pathology examination in which the following parameters were recorded: gravid uterus weight; number of corpora lutea, implantations, resorptions (early [only placenta visible] and late [placenta and embryo visible]); and individual placental weight. A necropsy was performed on those dams that died during the study in order to determine the cause of death. Corpora lutea and implantations were counted, when possible, and organs with gross alterations were fixed in formalin for histologic examination, if necessary. The uteri of non-pregnant females were stained using the Salewski[1] method and examined for the presence of early resorption sites.

[1] Salewski E. 1964. Färbemethode Zum Makroscopichen Nachweis von Implantationsstellen am uterus der Ratte. Arch. Exp. Path. Pharmak. 247:367.

2. <u>Fetal Evaluations</u>: The method used to sacrifice fetuses is not provided in the study report. Individual fetal weights, the number and sex of viable fetuses, and the number and sex of dead fetuses (fetuses without spontaneous movements and breathing) were recorded. A gross examination was performed on all fetuses immediately. Those showing external malformations were fixed in order to preserve the evidence of malformation. Skeletal malformations, anomalies, and variants (defined below) were determined by clearing half of the fetuses per litter and staining them with Alizarin Red S. Those with suspected skeletal malformations were cleared after a preliminary observation. The remaining fetuses per litter were preserved in Bouin's fluid and examined using the Wilson[2] technique. When possible, the distribution per litter for examination by clearing or by Wilson's technique was equal by sex. Abnormalities were classified as malformations, anomalies, and variants and were defined as follows:

- Malformations: rare and/or usually lethal (e.g., hydrocephaly, thoracocele, acephalia, amelia, phocomelia, celosomia, etc.);
- Anomalies: more frequent and not lethal (e.g., reduced cranial ossification, hemorrhages, etc.);
- Variants: common in the control populations and often definable only in terms of continuous variable gradients (e.g., poor ossification of sternebrae, pubis, or other).

D. <u>DATA ANALYSIS</u>

[2] Wilson JG. 1965. Embryological considerations in teratology. In Wilson JG and Warkany J (eds.). Teratology: Principles and Techniques. University of Chicago Press: Chicago.

1. <u>Statistical analyses</u>: Group mean values were calculated from individual data in two ways: (1) calculated for all surviving females that showed signs of pregnancy, including those presenting 100% of post-implantation losses; and (2) calculated only for those females with viable fetuses at term. To compare frequency data, the heterogeneity test[3] (Chi square 2xN) and Fisher's exact test[5] were applied. The Trend test[4] also was applied and was denoted in summary tables next to the appropriate groups with the word "TREND." All these tests were one-tailed. All other continuous data were evaluated for homogeneity by using Bartlett's homogeneity of variance. Homogenous parameters were then evaluated by an ANOVA[5]; those values reaching statistical significance ($p<0.05$) were further evaluated through Dunnett's multiple comparison test[5]. Non-homogenous parameters were transformed (by applying the inverse, logarithm decimal, square, and/or square root) and then re-evaluated with Bartlett's homogeneity of variance. Homogenous parameters were evaluated as before, (i.e., by using an ANOVA and a Dunnett's multiple comparison test for those values that reached statistical significance [$p<0.05$]). Those that remained non-homogenous were evaluated by a Kruskal-Wallis non-parametric ANOVA. Significant ($p<0.05$) findings were further evaluated through the Mann Whitney's U test[6].

2. <u>Indices</u>: The fertility index was defined as the percent ratio between the number of females having evident signs of pregnancy with respect to the number of females that had positive vaginal smears. In addition, pre- and post-implantation loss were calculated as follows:

Pre-implantation loss = ([number of corpora lutea - number of implantations]/number of corpora lutea) X 100

Post-implantation loss = ([number of implantations - number of viable fetuses]/number of implantations) X 100

3. <u>Historical control data</u>: Historical control data are presented in Attachment 2 of the study report.

II. RESULTS

[3] Armitage P. 1971. Statistical Methods in Medical Research. Blackwell Scientific Trend Publication.

[4] International Agency for Research on Cancer (IARC). 1980. Long-term and short-term screening assay for carcinogens: A critical appraisal. IARC Monographs (Suppl. 2):386-388.

[5] Dunnett CW. 1955. J. Am. Statist. Assoc. 50:1096-1121.

[6] Siegel S. 1956. Non-parametric Statistics for Behavioral Sciences. McGraw Hill: New York.

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: All dams survived until the scheduled necropsy. There were no treatment-related clinical signs or behavioral changes noted in any group.

2. Body Weight: Body weight, body weight gain, and corrected body weight gain are summarized in Tables 2, 3, and 4. At 1000 mg/kg/day, there was a significant decrease in mean body weight from gd 10 until the end of the study. Body weight gain in the 1000-mg/kg/day group also was significantly decreased during treatment (gd 6-15 as well as gd 6-10 and gd 10-15). Although body weight gain in the 1000-mg/kg/day group following treatment (gd 15-20 as well as gd 15-18 and gd 18-20) was comparable to or even exceeded the control, overall body weight gain for the study (gd 0-20) was still significantly decreased. Corrected body weight and corrected body weight gain (gd 6-20) also were significantly decreased at 1000 mg/kg/day. At 200 mg/kg/day, there was a significant decrease in body weight gain during treatment (gd 6-10 and gd 6-15), as well as a significant decrease from gd 6-18. Also, there was a significant decrease in corrected body weight gain when calculated from gd 6-20. At 40 mg/kg/day, body weight and body weight gains were similar to the control groups, except for a statistically significant decrease in body weight gain from gd 6-10 and a statistically significant increase in body weight gain post-treatment from gd 18-20. Corrected body weight and corrected body weight gains at the low dose were comparable to the control group.

TABLE 2. Mean (±SD) Maternal Body Weight (g) [a]

Gestation Day	Dose in mg/kg bw/day (# of Dams)			
	Control (23)	40 (21)	200 (24)	1000 (23)
0	286.74±22.169	291.71±27.809	289.75±25.290	291.91±22.358
6	320.26±22.114	327.76±27.880	326.54±23.659	327.13±23.412
10	337.83±24.028	341.38±25.427	331.75±23.382	317.96*±21.582
15	367.57±27.786	373.62±24.937	356.88±23.901	331.61**±28.202
18	407.22±30.667	415.24±29.021	399.96±31.109	370.78**±34.893
20 Gestation Day	438.00±37.093	453.52±29.978	430.21±37.104	406.65*±42.352

a Data obtained from page 38 in the study report. * Statistically different (p <0.05) from the control.
** Statistically different (p <0.01) from the control.

TABLE 3. Mean (±SD) Maternal Body Weight Gain (g) [a]

Interval	Dose in mg/kg bw/day (# of Dams)			
	Control (23)	40 (21)	200 (24)	1000 (23)
Pre-treatment Days 0-6	33.52±10.286	36.05±6.903	36.79±12.918	35.22±7.367
Treatment: Days 6-10	17.57±6.618	13.62*±6.344	5.21***±9.459	-9.17***±11.384
Days 10-15	29.74 ±8.729	32.24 ±12.304	25.13 ±7.925	13.65** ±11.097
Days 6-15	47.30±11.129	45.86±14.354	30.33**±13.328	4.48**±12.548
Post-treatment: Day 15-18	39.65±7.253	41.62±15.068	43.08±11.736	39.17±13.710
Day 18-20	30.78±11.897	38.29*±7.191	30.25±10.816	35.87±14.867
Days 15-20	70.43±16.572	79.90±15.881	73.33±19.524	75.04±21.094
Other Study Intervals: Days 6-18	86.96±15.616	87.48±16.161	73.42*±20.149	43.65**±21.174
Days 6-20	117.74±24.653	125.76±19.442	103.67±27.234	79.52**±28.813
Days 0-20	151.26±24.057	161.81±19.577	140.46±24.606	114.74**±27.539

a Data obtained from pages 39-41 in the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.
*** Statistically different ($p < 0.001$) from the control.

TABLE 4. Mean (±SD) Maternal Corrected Body Weight and Corrected Body Weight Gain (g) [a]

	Dose in mg/kg bw/day (# of Dams)			
	Control (23)	40 (21)	200 (24)	1000 (23)
Corrected BW	351.65±24.918	353.70±28.998	343.21±28.541	326.04**±27.367
Corrected BW Gain Days 6-20	31.39±10.961	25.94±12.747	16.67**±16.685	-1.09**±13.024
Corrected BW Gain Days 0-20	38.46±20.048	34.10±26.343	33.29±22.676	23.89±15.185

a Data obtained from pages 38-41 in the study report.
** Statistically different ($p < 0.01$) from the control.

3. <u>Food Consumption</u>: Food consumption data are summarized in Table 5. Significant decreases in food consumption were observed during treatment in the high- and mid-dose groups (gd 6-10 and gd 10-15). Food consumption for the low-dose group was comparable to the control group.

TABLE 5. Mean Food Consumption (g/animal/day) (±S.D.) [a]

Interval	Dose in mg/kg bw/day (# of Dams)			
	Control (23)	**40 (21)**	**200 (24)**	**1000 (23)**
GD 0-6	23.26±2.736	23.51±2.643	23.85±2.506	24.16±1.666
GD 6-10	23.71±3.106	22.14±2.453	18.71**±2.271	14.58**±3.613
GD 10-15	25.60±2.655	24.74±2.640	23.02*±3.869	18.90**±3.736
GD 15-18	27.30±3.690	28.37±3.633	28.64±7.222	25.06±4.340
GD 18-20	29.04±7.327	29.76±3.823	27.50±5.707	31.67±12.755

a Data obtained from page 42 in the study report.
* Statistically different (p <0.05) from the control.
** Statistically different (p <0.01) from the control.

4. <u>Gross Pathology</u>: The study does not report any gross pathology findings for any of the dams; there were no treatment-related abnormalities found.

5. <u>Cesarean Section Data</u>: Cesarean section data are summarized in Table 5. In general, observations made in treated animals during the cesarean section examination were comparable to the control group. At 1000 mg/kg/day, there was a significant increase in the total number of dead fetuses, as well as a significant decrease in mean placental weight when compared to the control group. The increase in the total number of dead fetuses was attributed to one high-dose dam. At 40 mg/kg/day, there was a significant increase in the mean number of implantations per dam, as well as a significant increase in the mean number of live fetuses per dam, when compared to the control group. According to the study author, the increases in the mean number of implantations per dam and in the mean number of live fetuses per dam were not considered treatment-related because the values were considered normal for the strain of rat used and a dose-response relationship was not present (i.e., no significant effect in the mid- or high-dose groups).

TABLE 5. Cesarean Section Observations [a]

Observation	Dose (mg/kg bw/day)			
	0	**40**	**200**	**1000**

Observation	Dose (mg/kg bw/day)			
	0	40	200	1000
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant	23	21	24	23
Pregnancy Rate (%)	92	84	96	92
# Nonpregnant	2	4	1	2
Maternal Wastage				
# Died	0	0	0	0
# Died Pregnant	0	0	0	0
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Placental Weight (g)	0.54±0.090	0.49±0.066	0.51±0.126	0.45**±0.081
Total # Corpora Lutea	404	410	444	447
Corpora Lutea/Dam	17.57±4.388	19.52±2.562	18.50±3.362	19.43±4.521
Total # Implantations	342	379	394	380
(Implantations/Dam)	14.87±4.003	18.05*±3.201	16.42±4.393	16.52±4.032
Total # Litters	23	21	24	23
Total # Live Fetuses	326	368	371	352
(Live Fetuses/Dam)	14.17±4.324	17.52*±2.892	15.46±4.283	15.30±4.617
Total # Dead Fetuses	0	0	0	7*
(Dead Fetuses/Dam)	0.0±0.000	0.0±0.000	0.0±0.000	0.30±1.460
Total # Resorptions	16	11	23	21
Early	16	10	23	20
Late	0	1	0	1
Resorptions/Dam	0.70±1.146	0.52±0.981	0.96±1.398	0.91±1.083
Early	0.70±1.146	0.48±0.981	0.96±1.398	0.87±1.100
Late	0.00±0.000	0.05±0.218	0.00±0.000	0.04±0.209
Litters with Total Resorptions	0	0	0	0
Mean Fetal Weight (g)	3.96±0.687	3.67±0.361	3.60*±0.455	3.16**±0.408
Males	4.09±0.750	3.79±0.384	3.73±0.469	3.27**±0.443
Females	3.86±0.647	3.57±0.373	3.44*±0.406	3.05**±0.390
Sex Ratio (% Male)	49.08	48.64	49.60	52.27
Pre-implantation Loss (%)	13.87±15.820	7.66±12.419	12.83±20.163	13.32±17.773
Post-implantation Loss (%)	5.36±10.912	2.55±4.682	5.43±8.077	8.17±12.615

a Data obtained from pages 43-48 in the study report.
* Statistically different (p <0.05) from the control.
** Statistically different (p <0.01) from the control.

B. DEVELOPMENTAL TOXICITY

1. External Examination: Mean litter weights and sex ratios are presented in Table 5 above. All sex ratios were comparable. There was a significant decrease in mean fetal weight and mean female fetal weight at 1000 and 200 mg/kg/day; however, according to the study author, only values observed at 1000 mg/kg/day were lower than the historical control mean values (±S.D.) (3.677±0.334 combined, 3.765±0.344 male, and 3.580±0.315 female). Additionally, at 1000 mg/kg/day, there was a significant decrease in mean male fetal weight. At 40 mg/kg/day mean fetal weights (combined, male, and female) were comparable to the control.

The incidences of external variants, anomalies, and malformations are provided in Table 6a below. There were no treatment-related external variants, anomalies, or malformations.

2. Visceral Examination: The incidences of visceral variants, anomalies, and malformations are provided in Table 6a below. There were no treatment-related visceral variants, anomalies, or malformations.

3. Skeletal Examination: The incidences of skeletal variants, anomalies, and malformations are provided in Table 6b below. There were no treatment-related skeletal anomalies or malformations. There was a higher number of skeletal variants, specifically a higher incidence of sternum variants, observed in the high-dose group as compared to the control group. Also, an increase in the mean number and percent of fetuses with skeletal variants was observed at the high-dose level. According to the study author, these values (the incidence, mean, and percent) fell outside of the historical control values. Additionally, there was a significant increase in the incidence of skeletal variants in the mid- and low-dose groups; however, according to the study author, these differences were not considered treatment-related because the values fell within the historical control values.

TABLE 6a. Incidence of External and Visceral Variants, Anomalies, and Malformations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0	**40**	**200**	**1000**
External				
#Fetuses (litters) examined	326 (23)	368 (21)	371 (24)	352 (23)
#Fetuses (litters) with anomalies	0 (0)	0 (0)	1 (1)	2 (2)
#Fetuses (litters) with malformations	0 (0)	1 (1)	0 (0)	2 (2)
Visceral				
#Fetuses (litters) examined	164 (23)	183 (21)	189 (24)	174 (23)
#Fetuses (litters) with variants	23 (15)	27 (14)	31 (12)	20 (13)
#Fetuses (litters) with anomalies	0 (0)	0 (0)	1 (1)	1 (1)
#Fetuses (litters) with malformations	0 (0)	0 (0)	1 (1)	0 (0)

a Data obtained from pages 49-50 and 55-56 in the study report.
b Some observations may be grouped together.

TABLE 6b. Incidence of Skeletal Variants, Anomalies, and Malformations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0	**40**	**200**	**1000**
#Fetuses (litters) examined	164 (23)	185 (21)	183 (23)	176 (23)
#Fetuses (litters) with variants	128 (22)	164** (21)	157* (22)	174*** (23)
Sternum variants [c]	128 (22)	164 (21)	157 (22)	174 (23)
#Fetuses (litters) with anomalies	24 (14)	23 (12)	33 (17)	31 (16)
#Fetuses (litters) with malformations	0 (0)	0 (0)	0 (0)	0 (0)

a Data obtained from pages 49-50 and 55-57 in the study report.
b Some observations may be grouped together.
c Fetal (litter) incidence
* Statistically different (p <0.05) from the control.
** Statistically different (p <0.01) from the control.

TABLE 6c. Mean Number and Percent of Fetuses with Skeletal Variants [a]

Observations	Dose (mg/kg bw/day)				
	Historical Control	0	40	200	1000
Mean No.	6.130±1.963	5.57±2.428	7.81**±1.601	6.54±2.686	7.57**±2.273
Mean %	87.220	76.69±28.349	89.03±12.674	85.41±23.122	98.76***±5.958

a Data obtained from pages 51, 53, and 182-183 in the study report.
** Statistically different (p <0.01) from the control.
*** Statistically different (p <0.001) from the control.

III. DISCUSSION and CONCLUSIONS

1. <u>Maternal toxicity</u>: Maternal toxicity was observed at 1000 and 200 mg/kg/day based on decreased body weight, body weight gain, corrected body weight gain, and/or food consumption.

Therefore, the maternal LOAEL is 200 mg/kg/day (based on decreased body weight gain, corrected body weight gain, and food consumption), and the NOAEL is 40 mg/kg/day.

2. <u>Developmental toxicity</u>:

a. Deaths/Resorptions: Fetal deaths at the 1000 mg/kg/day dose showed statistical significance compared to the control (Table 5). This deaths, however, occurred in the pups of only one dam and was not considered to be of any toxicological importance. Resorptions observed in the study were comparable to the control group.

b. Altered Growth: Mean fetal weights (combined, male, and female) were significantly lower at 1000 mg/kg/day. Decreased mean fetal weights (combined and female) also were observed at 200 mg/kg/day; however, these values fell within the mean (±S.D.) historical control values.

c. Developmental Variations: There were no treatment-related external or visceral variations. There was a higher number of skeletal variants, specifically a higher incidence of sternum variants, observed in the high-dose group as compared to the control group. Also, an increase in the mean number and percent of fetuses with skeletal variants was observed at the high-dose level. According to the study author, these values (the incidence, mean, and percent) fell outside of the respective historical control values. Additionally, there was a significant increase in the incidence of skeletal variants in the mid and low-dose group. According to the study author, these values fell well within the historical control values and were not considered treatment-related. Our reviewers disagree, noting that the mean number of 40-mg/kg/fetuses with skeletal variants also exceeded the mean historical control value; however, we do not believe that this effect is treatment-related because values at 200 mg/kg/day were within mean historical control values and, therefore, a dose-response relationship could not be established.

d. Anomalies/Malformations: There were no treatment-related external, visceral, or skeletal anomalies or malformations observed in the study.

Overall, the developmental effects were mainly observed at 1000 mg/kg/day. **Therefore, the developmental LOAEL is 1000 mg/kg/day (based on decreased fetal weight and increased incidence of skeletal variations), and the NOAEL is 200 mg/kg/day.**

IV. STUDY DEFICIENCIES: There were no major study deficiencies.

V. STUDY CLASSIFICATION: This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a developmental toxicity study (OPPTS 870.3700 [§83-3a]; OECD 414) in rats.

Sign-off Date : 06/08/05
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